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Safety and Immunogenicity of a Live Attenuated Pentavalent Rotavirus Vaccine in HIV-Exposed Infants With or Without HIV Infection in Africa

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Conflicts of Interest

MJ Levin (chair), JC Lindsey (statistician), W Schimana (vice chair), MM McNeal (laboratory), B Heckman (data manager), P Sato (NIAID medical officer), GK Siberry (NICHD medical officer), GA Weinberg (investigator), and A Weinberg (immunologist) were members of the core protocol team supported by research grants.

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Abstract

Objective—Although many HIV-infected (HIV+) and HIV-exposed but uninfected (HEU) infants have received live rotavirus vaccines since the World Health Organization recommended universal administration of these vaccines to infants, there has been limited prospective information on their safety and immunogenicity in either group of infants.

Design/Methods—We performed a randomized, double-blinded, placebo-controlled trial of the safety and immunogenicity of oral pentavalent rotavirus vaccine (RV5) administered to HIV+ and HEU infants in 4 African countries. Ninety-three % of HIV+ infants were receiving antiretroviral therapy prior to vaccination. Participants were followed for safety. Immune responses were measured 14 days after three doses of RV5, including serum anti-rotavirus neutralizing and IgA antibodies; IgA antibody in stool; and anti-rotavirus memory B- and T-cell Fluorospot. Shedding of RV5 in stool was monitored.

Results—76 HIV+ and 126 HEU infants were enrolled from 2009-2013. No significant differences were found in adverse event rates, including grade 3 events, between RV5 and placebo recipients, for either HIV+ or HEU infants. The proportion of anti-rotavirus IgA responders (≥3-fold increase from baseline) after RV5 administration was 81% in both HIV+ and HEU infants, which was approximately 2.5-fold higher than in placebo recipients ($p<0.001$). Neutralizing antibody responses to 3 of 5 serotypes were significantly higher after RV5 regardless of HIV status, and those of HIV+ infants were equal or greater than responses of HEU infants to all 5 serotypes. Only one HIV+ RV5 recipient had RV5 isolated from stool.

Conclusion—RV5 was immunogenic in both HIV+ and HEU infants and no safety signals were observed.

Keywords

HIV exposed; HIV infection; infants; rotavirus vaccine; safety; immunogenicity; rotavirus

A. Introduction

Rotavirus is a major cause of infant diarrheal morbidity and mortality world-wide [1,2]. Live attenuated rotavirus vaccines (RVs) reduce rotavirus-related disease in healthy children in resource-rich and resource-limited countries [3-5]. Diarrheal disease is a major cause of sickness and death in HIV-infected (HIV+) children; some studies report that rotavirus infection is more severe in HIV+ children [5-9]. Although many HIV+ infants have received

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live RVs since the WHO recommendation for these vaccines, the efficacy of RVs for HIV+ infants has not been determined [10-12]. Information on the safety and immunogenicity of RVs in HIV+ infants is limited to approximately 100 infants who received the monovalent RV (Rotarix™, GlaxoSmithKline; RV1) [12,13] and <50 infants who received the pentavalent RV (RotaTeq™, Merck & Co., Inc.; RV5) [14,15]. Additional information about RVs in HIV+ infants is desirable because protective antibody responses can be impaired in infants with untreated HIV infection [16-19], and robust responses may not be achieved even when vaccine is administered after initiating antiretroviral therapy (ART) early in life [18,20-22]. This may be more problematic in resource-poor countries where RVs induce lower titers of rotavirus-specific antibody and vaccine efficacy is lower than in resource-rich countries [23]. Moreover, while HIV+ infants may benefit from RVs, these vaccines have been implicated in prolonged gastroenteritis with persistent shedding of vaccine-strain virus in infants with severe immune deficiency, and other live viral vaccines have caused disease in children with advanced HIV infection [24-27]. Information about rotavirus vaccination of infants who are exposed to HIV, but not infected (HEU), is also desirable, since HEU infants have an excess of infectious morbidity during the first year of life [28,29]. Although HEU infants make normal levels of antibody to some vaccines typically administered during infancy [30], information on the immunogenicity and safety after administration of RVs to HEU infants is important, given the large number of infants born to HIV-infected women.

The current report describes a randomized, placebo-controlled trial comparing the safety and immunogenicity of RV5 in HIV+ and HEU infants.

B. Methods

1. Study design

This study (P1072) sponsored by the International Maternal Pediatric Adolescent AIDS Clinical Trials (IMPAACT) network was a Phase II randomized double-blind study of RV5 in infants born to HIV+ mothers (NCT00880698). It was approved by Institutional Review Boards of IMPAACT and appropriate institutions or national governments. Parental consent was obtained. P1072 was conducted in 4 African countries where RV was not in the national vaccination program. Infants between 2 and <15 weeks old at screening were determined to be HEU or in one of three HIV+ strata (details in *Supplemental Information*). Infants in each stratum were randomized to receive RV5 or placebo: study dose 1 at 4 to <15 weeks; and study doses 2 and 3 at ≥28 days after the previous vaccination, with dose 3 by ≤32 weeks. Participants were followed until six weeks after the last dose, with visits at 7, 14, 21, and 42 days after each dose to record clinical signs, symptoms and new significant diagnoses. No clinical laboratory testing was required, but sites recorded laboratory results considered pertinent. Stool samples were collected at entry; at days 7, 14, 21, and 42 after dose 1; at days 7 and 21 after doses 2 and 3; and at unplanned visits for gastroenteritis. Blood for immunogenicity testing was collected at entry and 14 days after dose 3 (42 days if not collected at 14 days).

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2. Study conduct

Shortly after the study began the protocol was amended to require HIV+ infants to receive ART before receiving study vaccine. Six of 76 (7%) of these infants received study vaccine prior to this requirement. Enrollment was closed in participating countries when RV1 was added to national vaccine schedules (details in *Supplemental Information*).

3. Study outcomes

Safety—Laboratory values, signs, symptoms and diagnoses were graded according to the Division of AIDS Table for Grading Severity of Adult and Pediatric Adverse Events [31]. Sites reported grade ≥1 signs, symptoms, and diagnoses. Events that were grade ≥2, and grade ≥1 targeted signs/symptoms (vomiting, fever, diarrhea, irritability), targeted diagnoses (gastroenteritis, intussusception, and diagnoses with the rotavirus organism code), and deaths were reviewed by the Core Team (study chairs, immunologist, NIH medical officers, and pharmaceutical representatives).

Immunogenicity

Serum anti-rotavirus neutralizing antibodies (SNA): Neutralizing antibodies to type-specific outer surface proteins of RV5 (G1, G2, G3, G4, and P1A) were measured as published [32] (details in *Supplemental Information*).

Serum anti-rotavirus IgA antibody: was measured by a standard EIA format previously published [33] (details in Supplemental Information).

Copro-antibody (stool anti-rotavirus IgA): was measured in stool filtrates by the methods used for serum IgA, but standardized to total IgA and reported as rotavirus antigen units/μg of total IgA.

Anti-rotavirus memory B and T cell responses (FluoroSpot)

Memory B cell responses: Peripheral blood mononuclear cells (PBMC) were cryopreserved at clinical sites using a standardized protocol [34] and shipped for detection of IgG/IgA secreting cells (SC) (details in *Supplemental Information).*

T cell responses: A dual color IFNγ and IL2 ELISPOT assay (ELISPOT MabTech FluoroSpot kit) was used per manufacturer's instructions (details in *Supplemental Information).*

Shedding of rotavirus in stool: Rotavirus in stool was initially assessed using an ELISA assay using a published commercial rotavirus antigen detection kit [35]. Positive samples were identified as vaccine or wild type with RT-PCR assays specific for rotavirus VP4, VP6, and VP7 genotypes, and infectious virus identified with a fluorescent focus assay (FFA) [35].

Statistical methods

Baseline and safety data were presented ‘as-randomized’ (intent-to-treat). Immunogenicity analyses were conducted in the ‘per-protocol’ population, defined as participants completing

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the three as-randomized vaccinations within the required windows. Sensitivity analyses were done for safety, only including participants who received the correct vaccine, and for immunogenicity, in the intent-to-treat population. Proportions were presented with exact 95% confidence intervals (CI) and compared using Fisher's exact tests (unadjusted) and logistic regression (adjusted). Continuous outcomes were compared using Wilcoxon rank sum tests (unadjusted) and censored normal regression on log10-transformed levels adjusted for other covariates.

Safety—Proportions of participants experiencing new adverse events (appearing after the first vaccination, or of increased grade reported after the first vaccination) were presented by HIV-1 stratum and vaccine group.

Immunogenicity

Serum antibodies: Measurements outside the lower or upper limits of quantitation (LLOQ/ULOQ) of each assay were set to those limits. The primary outcome measure was pre-defined as ≥3-fold increase achieved post-dose 3 (PD3) in SNA and IgA over the entry value. If the entry value was above one third of the ULOQ, the infant was not classified as a responder and was excluded from analysis. A secondary outcome was antibody level achieved PD3.

B and T-cell responses: IgA and IgG memory B cells were measured in samples from a randomly chosen subset that received three vaccinations and met viability assay criteria. Each response was measured in duplicate wells at entry, 14, and 42 days PD3. If two measurements were available, the analysis unit was the mean of both responses. T-cell responses were calculated as the difference between WC3-containing wells and the MA104 controls. Negative differences and differences of zero were set to 0.1 in order to allow log transformation for analysis. Participants were classified as responders if they achieved a ≥2-fold increase over entry levels by either 14 or 42 days PD3.

Copro-antibodies: Measurements below the LLOQ were set to the LLOQ. Spearman correlations were calculated on PD3 levels of copro-antibodies and serum IgA antibodies.

Vaccine virus in stool: Numbers of infants with EIA-positive stool after each study dose were reported. The EIA-positive samples that were FFA+ and RT-PCR+ for VP6 were summarized.

Analyses were conducted in Statistical Analysis System (SAS) Version 9.4.

C. RESULTS

Accrual and baseline characteristics

Between December 2009 and October 2013, 202 infants (126 HEU; 76 HIV+) were enrolled [(79% of the target for HEU; 48% for HIV+ (Table 1)]. HIV+ infants were less likely to have received prophylaxis to prevent HIV transmission (PMTCT) and their mothers were less likely to have received ART. The CONSORT diagram (Supplemental Figure 1) indicates the number of participants in each treatment arm; 188 (93%) received study vaccine per

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protocol. Accrual was low in Tanzania because of late approval of the study by the national review board and in Zambia and Tanzania because of early adoption of a national recommendation for rotavirus vaccination.

Safety

Adverse events and targeted signs/symptoms are summarized in Table 2. Proportions of participants with adverse events tended to be higher in HIV+ infants, but this was true regardless of exposure to RV5. There was no statistically significant difference in proportions of HIV+ or HEU participants receiving either RV5 or placebo, or within CD4% strata for the HIV+ infants (Table 2; Supplemental Tables 1 and 2). Event rates within seven days of vaccination were similar. CD4% increased and HIV RNA viral load decreased significantly in HIV+ infants from entry to study end in both RV5 and placebo recipients, with no differences in the magnitude of change in CD4% or proportions of participants with HIV-1 RNA ≤400 copies/ml between vaccine groups.

Three HIV+ infants (1 RV5; 2 placebo) died of pneumonia 3-4 weeks after the first study dose. These were deemed by the site and Core Team as not, or probably not, related to study vaccine. Eight HIV+ infants (5 RV5; 3 placebo) and five HEU infants (2 RV5; 3 placebo) were hospitalized during the study. Reasons for hospitalization were: gastroenteritis (4), pneumonia (4), malaria (1), measles (1), febrile seizures (1); no diagnosis recorded (2). There were no statistically significant differences between RV5 and placebo in changes from baseline in WHO weight- or height-for-age z-scores. HIV test results were available for 121/126 HEU infants ≥ 14 days PD3; all were negative.

Rotavirus Serum Antibody Responses

The proportion of anti-rotavirus IgA responders (≥3-fold increase from baseline) reached 81% in both HIV+ and HEU recipients of RV5 and was approximately 2.5-3-fold higher than in placebo recipients (Table 3). Response rates in the SNA assay varied by serotype (~20% to 60%). Proportions of RV5 recipients responding to each serotype was consistently higher, for both HIV+ and HEU infants, compared to placebo recipients. For all SNA assays except P1A, the proportion of RV5 responders was higher in HIV+ compared to HEU infants. This was likely due to higher levels of transplacentally-transferred maternal antibodies in HEU infants, which limited their ability to achieve a 3-fold increase in SNA antibody after vaccination. This is demonstrated in Table 4 and Supplemental Figure 2, which show that median levels for each specific antibody were higher in the HEU infants at baseline ($p \leq 0.001$ for all SNA). Antibody levels were consistently higher in RV5 recipients, for both HIV+ and HEU infants, compared to placebo recipients. Importantly, both IgA and SNA post-vaccination antibody levels were not significantly different by HIV status (Table 4 and Supplemental Figure 2).

Adjusted analyses were performed to identify potential predictors of response and of levels achieved PD3 among RV5 recipients. Covariates included: (i) infant ever breastfed , (ii) oral polio vaccine (OPV) co-administered with the first or with three vaccinations, (iii) infant exposure to prophylaxis to prevent PMTCT , and (iv) any detection of rotavirus antigen in stool between the first and last doses. For HIV+ infants, additional covariates included: (i)

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screening CD4%, (ii) entry HIV-1 RNA, (iii) infant exposure to PMTCT, and (iv) number of days on ART at entry. Adjusted analyses for each of these factors had little effect on the magnitude or statistical significance of the odds of responding to RV5 relative to estimates from unadjusted models (data not shown). No covariates were consistently associated with the odds of responding or with PD3 levels across immunologic assays. Because of the number of models fit and the lack of consistent findings across outcomes, we do not report the few statistically significant findings.

Rotavirus Copro-antibodies

Median (Q1, Q3) levels of copro-antibodies at entry were higher in HIV+ than in HEU infants, but the difference was not statistically significant (p=0.13) (Table 4). Post-vaccination levels were significantly higher in RV5 recipients compared to placebo-recipients in HEU, but not HIV+ infants. Copro-antibody levels were not significantly different in RV5 recipients between the HIV+ and HEU infants. Copro-antibody and serum antibody levels PD3 were positively correlated (Spearman correlation = 0.55 for HEU [p<0.001]; 0.39 for HIV+ [p=0.040]).

Cellular immunity

At entry, RV-specific B and T cell immunity were very low (median=0.1; Table 5) and did not differ by HIV status. After vaccination, IgA B cell memory was significantly higher in HIV+ infants receiving RV5 compared with placebo recipients (p=0.04; Table 5), although this was based on the distribution of values in the third quartile and the magnitude of the difference was small. There were no significant differences in HEU infants. IgG memory B cell responses did not appreciably increase after vaccination. There were no statistically significant differences in proportions of participants with a ≥2-fold increase in WC3-specific memory B or T cells at PD3 compared to entry, either by vaccine group within HIV status, or by HIV status in RV5 recipients (Table 5). These results should be interpreted with caution, because at entry most participants had no secreting T- or B-cells, so that small increases were considered a response. PHA responses were poor and did not differ between vaccine groups or by HIV status in RV5 recipients (data not shown).

Fecal shedding

All participants had at least one stool sample collected after the first vaccination (Supplemental Table 3). Nine of 99 (9%) RV5 recipients after the first dose, and 1 of 98 after the second dose, had at least one stool sample positive for rotavirus by EIA. Across all samples at all times rotavirus was detected by EIA in 13.0% (8 of 62; 1 positive after both dose 1 and 2) of the HEU infants and in 2.7% (1 of 37) of the HIV+ infants who received RV5; for placebo- recipients, these percentages were 6.3% (4 of 64) for the HEU and 7.7% (3 of 39) for the HIV+ infants.

All EIA-positive samples were evaluated by FFA to determine if infectious rotavirus was present, and by RT-PCR to determine the rotavirus source, and were further characterized by VP4 and VP7 type. Only one of 37 (2.7%) HIV+ RV5 recipients shed FFA+ vaccine-type rotavirus after the first vaccination. No shedding was detected in any infant after the third vaccination.

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D. DISCUSSION

There was no evidence that RV5 was associated with excess adverse signs or symptoms in either HIV+ or HEU infants. Two of 3 deaths in HIV+ infants occurred in placebo recipients. One death and 8 hospitalizations occurring in HIV+ infants were attributed to infectious causes common in these infants. Moreover, vaccination did not alter the CD4% or viral load response to ART in RV5 recipients during the study. Although limited in number, no HEU recipients of RV5 acquired HIV infection during the study. The prospective safety information collected is reassuring and consistent with previously published information for both RVs.

Virus-specific serum IgA antibody responses after RV5 vaccination were not significantly different in HIV+ and HEU infants, both in terms of 3-fold rise and post-vaccination titer. Of note, levels of serum IgA, which is not transplacentally transferred, did not differ before vaccination between HEU and HIV+ participants. This is important because serum IgA has been associated with protection against symptomatic disease and disease severity after natural exposure [36-39], and serum IgA correlated with protection in a study of an experimental rhesus rotavirus vaccine [33]. The magnitude of IgA antibody induced by RV5 in the current study was almost identical, utilizing the same laboratory assay, to that reported in a large trial of the safety and efficacy of RV5 in Africa [15]. A systematic review of RV trials in settings stratified by rate of childhood mortality (as a marker for medical and other resources) found that post-vaccination IgA antibody titer was lower in countries with higher childhood mortality and that titer correlated with lower efficacy [40]. In this context, our data suggest that the efficacy of the RV5 vaccine will not be lower in HIV+ or HEU compared with unexposed African infants, but also not as high as in infants in the United States.

Copro–antibodies were significantly induced by RV5 in HEU infants only, although there were no significant differences in PD3 levels between HIV+ and HEU infants after RV5 administration. The correlation of copro-antibodies with protection is less clear in adult challenge models and in relation to natural infection [41,42].

The assessment of SNA responses also demonstrated an increase in virus-specific antibody levels after RV5. These differed by antigen, and were especially strong against G1 and G4. Variable response by antigen was previously reported from United States and African efficacy studies, where G1 and G4 seroresponses were also most prominent [15,43]. SNA levels PD3 in RV5 recipients were not statistically different between HIV+ and HEU infants. SNA levels have also been associated with protection in clinical trials, including an analysis of 1857 subjects in Phase II/III trials of RV5 that correlated titers of SNA against G1 with protection against rotavirus gastroenteritis [41,44]. Most vaccinees were breastfed (63 %) and most received OPV (75% for dose 1; 59% for dose 2) concomitant with RV5. With the caveat of the limited sample size, we did not find that either of these interfered with the immune response to RV5, which is consistent with the published literature [45,46]. In addition, there was no discernible effect of entry CD4% or viral load on antibody responses.

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This is the first study of an orally administered live vaccine to HIV+ infants that stipulated ART prior to immunization. At the time of the first dose of RV5, 92% of the 37 HIV+ infants were receiving ART and only 1 had a CD4% <15. These were likely important factors in the responses to RV5, which significantly increased three different types of antibody utilizing three different laboratory methods. The second and third RV5 doses were administered after an interval of at least 1 and 2 months, respectively, of beginning ART, which may have contributed to the similar responses in both HIV+ and HEU infants. Moreover, where comparisons can be made, antibody titers after RV5 were similar to those reported in prior trials in unexposed and uninfected infants [15,43,44]. The paucity of shedding of RV5 after vaccination is additional evidence of immune preservation in our subjects.

HIV+ and HEU infants responded equally in all three immune assays. HEU infants were considered an appropriate proxy for healthy children, since the effectiveness of RV1 was the same in HEU and HIV-unexposed infants in a prior study [12]. However, the data on the adequacy of the HEU responses to vaccines is still mixed [30,47]. In the past, responses reported after childhood vaccinations were impaired in HIV+ infants, especially in those with low CD4%, high HIV viral load, and short duration of ART [17,19]. This is why current recommendations are to re-immunize HIV+ children who had been immunized before HIV therapy, but to delay this until 3 months after beginning ART [47,48]. This would not be feasible for RVs because of the need to provide protection early in infancy. The current study suggests that very early administration of RVs may be effective when given concomitant with ART. Furthermore, considering that in this study most infants received study vaccine at 80-90 days of life, and that they had only been on ART for a median of 4 days, the outcome measures might have been even better if ART was started earlier.

There is also evidence that the magnitude and duration of immune memory is impaired when immunization is attempted in severely immune suppressed HIV+ children, and that immune memory may be preserved when ART is started in infancy [47,49]. In this study, HIV+ RV5-recipients developed significantly higher numbers of IgA memory B cells compared with placebo recipients, although the magnitude of this difference was not large. Differences did not reach significance among HEU and there were no other statistically significant differences in PD3 levels of B- or T-cell mediated immune responses across vaccine- or HIV-status groups. Overall, cell-mediated immune responses to RV5 were of low magnitude. This was also true of non-specific responses, such as IFNγ and IL2 spot-forming cells after PHA stimulation, and total IgG and IgA secreting B cells (data not shown), suggesting that immaturity of the immune system contributed to the low cellular responses to RV5. In addition, other factors might have contributed to the low RV-specific cell-mediated immune responses, including homing to the gut in the immediate phase after immunization or acute RV infection as was observed in acute rotavirus infection [50]. Whether RV5 establishes persistent memory is an important question, since protection into the second year of life is essential, especially in resource-poor countries. Assuming that ART is started shortly after birth in HIV+ infants, as in this study, the third dose will be given after a long period of ART. This might influence persistence, as suggested by a report that a third dose of RV1, compared to the recommended two doses for that vaccine, resulted in higher serum IgA

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titers and significantly greater efficacy in the second year post-vaccination in a developing world setting [51].

The relatively small sample size of this study and the absence of an HIV-unexposed control group limit our ability to make definitive statements about RV5 in HIV-infected infants. Nevertheless, we found RV5 to be immunogenic in this placebo-controlled, randomized clinical trial and no safety signal was apparent. In the future, accurate assessment of the safety and value of RVs in HEU and HIV+ infants will require larger-scale effectiveness studies, since performing placebo-controlled efficacy trials will no longer be ethical.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Baseline Demographic Characteristics of Study Participants

Characteristic		HEU		HIV+	
		Placebo (N=64)	RV5[a] (N=62)	Placebo (N=39)	RV5[1] (N=37)
Country	Botswana[b]	18 (28%)	19 (31%)	16 (41%)	17 (46%)
	Tanzania	4 (6%)	3 (5%)	4 (10%)	2 (5%)
	Zambia	4 (6%)	4 (6%)	4 (10%)	2 (5%)
	Zimbabwe	38 (59%)	36 (58%)	15 (38%)	16 (43%)
Sex	Male	30 (47%)	29 (47%)	17 (44%)	18 (49%)
Age at randomization (days)	Median (Min, Max)	79 (30, 101)	82 (28, 103)	93 (64, 104)	92 (39, 101)
Ever breast fed at entry	Yes	43 (67%)	36 (58%)	26 (67%)	22 (59%)
	No	21 (33%)	26 (42%)	13 (33%)	15 (41%)
PMTCT[c]	No	6 (9%)	7 (11%)	12 (31%)	13 (35%)
	Yes	58 (91%)	55 (89%)	27 (69%)	24 (65%)
Mother receiving ARVs[d]	No	24 (38%)	25 (40%)	31 (79%)	31 (84%)
	Yes	40 (63%)	37 (60%)	8 (21%)	6 (16%)
ARVs (days) at entry[e]	Median (Min, Max)			2 (0, 50)	6 (0, 41)
WHO weight-for-age z-score	Median (Q1, Q3)	−0.6 (−1.3, −0.1)	−0.7 (−1.3, 0.0)	−1.2 (−2.5, −0.2)	−1.5 (−2.4, −0.5)
Screening CD4%	Median (Min, Max)	38 (19, 66)	37 (22, 62)	29 (7, 50)	31 (13, 58)
	<15%	0 (0%)	0 (0%)	2 (5%)	1 (3%)
	15% - <20%	1 (2%)	0 (0%)	4 (10%)	4 (11%)
	>=20%	63 (98%)	62 (100%)	33 (85%)	32 (86%)
Entry HIV-1 RNA (copies/ml)	Median			83,628	39,827
	< 10K			10 (27%)	12 (34%)
	10K - <100K			9 (24%)	9 (26%)
	100K - <750K			8 (22%)	7 (20%)
	>=750K			10 (27%)	7 (20%)
	Not measured			2	2

[a] RV5 = pentavalent rotavirus vaccine.

[b] Two sites.

[c] PMTCT = prevention of mother-to-child transmission.

[d] ARV = antiretroviral therapy.

[e] Six infants were not on ARVs when they received the first study vaccination.

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Table 2

Number of subjects with adverse events[a]

Adverse events	HIV status	Events/Total Placebo N=64	RV5[b] N=62	p-value[c]
	HEU	**N=64**	**N=62**	
	HIV+	**N=39**	**N=37**	
All ≥Grade 3[d]	HEU	3	1	0.62
	HIV+	5	5	1.00
Possibly/probably/definitely related ≥Grade 3[e]	HEU	1	1	1.00
	HIV+	1	1	1.00
All Grade 2	HEU	7	6	1.00
	HIV+	7	7	1.00
Possibly/probably/definitely related Grade 2[e]	HEU	2	4	0.44
	HIV+	2	2	1.00
Grade 1	HEU	20	25	0.35
	HIV+	16	19	0.49
Fever	HEU	12	16	0.39
	HIV+	15	11	0.47
Diarrhea	HEU	15	19	0.42
	HIV+	10	14	0.33
Vomiting	HEU	10	8	0.80
	HIV+	6	10	0.27
Irritability	HEU	1	1	1.00
	HIV+	0	1	0.49
Targeted diagnoses[f]	HEU	7	11	0.32
	HIV+	4	5	0.73

[a] Intent-to-treat population.

[b] RV5 = pentavalent rotavirus vaccine.

[c] Events graded according to Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events Version 1.0 [31].

[d] P-value comparing RV5-recipeints to placebo-recipients (Fisher's exact test).

[e] Relationship to study vaccine was assessed separately by the site and Core Team. In cases of non-agreement between the site and the Core Team, the assessment indicating the highest likelihood of causality was chosen for analysis.

[f] Targeted diagnoses = gastroenteritis, gastritis, intussusception, and any diagnosis with the rotavirus organism code.

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Table 3

Rotavirus antibody response rates 14 days post study dose 3 by HIV status and vaccine group[a]

Immunologic assay		Placebo		RV5[b]		Fisher's exact p-value	
	HIV-1 status	Responders[c]/N[d]	% (95% CI)	Responders[c]/N[d]	% (95% CI)	RV5:Placebo (within HIV strata)	HIV+:HEU (RV5 recipients)
Serum anti-rotavirus IgA							
	HEU	17/58	29 (18, 43)	46/57	81 (68, 90)	<0.001	1.00
	HIV+	5/31	16 (6, 34)	26/32	81 (64, 93)	<0.001	
Serum Neutralizing Antibody							
SNA[e] G1	HEU	1/58	2 (0, 9)	18/57	32 (20, 45)	<0.001	0.050
	HIV+	1/32	3 (0, 16)	18/34	53 (35, 70)	<0.001	
SNA[e] G2	HEU	3/58	5 (1, 14)	7/57	12 (5, 24)	0.20	0.24
	HIV+	3/32	9 (2, 25)	8/34	24 (11, 41)	0.19	
SNA[e] G3	HEU	1/58	2 (0, 9)	12/57	21(11, 34)	<0.001	0.45
	HIV+	0/32	0 (0, 119)	10/34	29 (15, 48)	<0.001	
SNA[e] G4	HEU	3/58	5 (1, 14)	18/57	32 (20, 45)	<0.001	0.008
	HIV+	1/32	3 (0, 16)	21/34	62 (44, 78)	<0.001	
SNA[e] P1	HEU	5/58	9 (3, 19)	14/56	25 (14, 38)	0.024	1.00
	HIV+	4/32	3 (4, 29)	8/33	24 (11, 42)	0.34	

[a] Per protocol population (received all 3 doses in required window).

[b] RV5 = pentavalent rotavirus vaccine.

[c] Responders defined as ≥ 3-fold rise from baseline to 14 days (at least 11 days) post dose 3. If pre-dose level > ULOQ/3 then excluded from analysis.

[d] N = number receiving RV5 or placebo.

[e] SNA - Serum neutralizing antibody.

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Table 4

Median (10^{th}, 90^{th} percentiles) rotavirus antibody levels at entry and post studydose 3[a]

Immunologic assay			Placebo				RV5[b]				Wilcoxon rank sum test		
	HIV status	Time point	N	Median	10th	90th	N	Median	10th	90th	HIV+:HEU (All at entry)[c]	HIV+:HEU (RV5)[d]	RV5:Placebo (within HIV- 1 strata)[e]
Serum anti-rotavirus IgA													
	HEU	Entry	58	1	1	25	59	2	1	35	0.08	0.55	
		PD3	58	3	1	257	58	66	5	1137			<0.001
	HIV+	Entry	33	2	1	86	35	2	1	127			
		PD3	33	2	1	267	35	119	7	625			<0.001
Serum Neutralizing Antibody													
SNA G1	HEU	Entry	58	38	13	154	59	42	15	130	0.001	0.92	
		PD3	58	18	10	48	58	53	14	452			<0.001
	HIV+	Entry	33	24	12	91	35	25	12	95			
		PD3	33	15	10	28	35	61	14	324			<0.001
SNA G2	HEU	Entry	58	48	16	139	59	62	21	135	<0.001	0.33	
		PD3	58	21	10	105	58	35	11	167			0.015
	HIV+	Entry	33	29	11	100	35	25	10	118			
		PD3	33	23	10	73	35	23	10	749			0.35
SNA G3	HEU	Entry	58	17	10	200	59	28	10	164	<0.001	0.33	
		PD3	58	10	10	40	58	22	10	159			<0.001
	HIV+	Entry	33	13	10	45	35	12	10	40			
		PD3	33	10	10	12	35	15	10	105			<0.001
SNA G4	HEU	Entry	58	55	19	152	59	67	16	178	<0.001	0.65	
		PD3	58	24	12	101	58	84	26	297			<0.001
	HIV+	Entry	33	27	12	82	35	17	10	56			

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Immunologic assay			Placebo				RV5[b]				Wilcoxon rank sum test		
	HIV status	Time point	N	Median	10th	90th	N	Median	10th	90th	HIV+:HEU (All at entry)[c]	HIV+:HEU (RV5)[d]	RV5:Placebo (within HIV- 1 strata)[e]
		PD3	33	15	10	62	35	101	15	464			<0.001
SNA P1	HEU	Entry	58	67	13	459	59	87	13	311	<0.001	0.11	
		PD3	58	25	10	263	58	60	16	539			0.003
	HIV+	Entry	33	31	10	200	35	32	11	182			
		PD3	33	12	10	145	35	34	10	645			0.015
Rotavirus IgA Copro-antibody													
	HEU	Entry	49	4.8	0.4	204.5	54	3.2	0.4	83.3	0.13	0.68	0.037
		PD3	55	3.5	0.4	60.4	51	15.6	0.7	88.6			
	HIV+	Entry	25	6.5	0.4	69.7	26	7.6	0.4	56.1			0.31
		PD3	26	5.6	0.4	211.4	28	10.8	1.3	192.4			

Lower and upper limits for serum anti-rotavirus IgA: 1.152 and 1250 (dilution=20).

Lower and upper limits for each serum neutralizing antibody (SNA): 10 and 1280 (dilution=2).

Lower limit for rotavirus IgA copro-antibody assay: 0.35 units/ml.

Results were similar when the five participants with pre-dose values greater than 1/3ULOQ were classified as non-responders and when they were classified as responders and included in the analysis.

[a] Per protocol population.

[b] RV5 = pentavalent rotavirus vaccine.

[c] Comparison of pre-entry levels by HIV status.

[d] Comparison of postdose 3 levels by HIV status in RV5 recipients.

[e] Comparison of postdose 3 levels within HIV stratum between RV5 and placebo.

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Table 5

RV5-Specific B-Cell and T-Cell Responses after Study Dose 3

Immune Response		HIV Status	≥2-fold increase % (N[a])		Post-dose 3Level median (Q1, Q3)	
			Placebo	RV5[b]	Placebo	RV5[c,d]
B-cell	IgA	HIV+	11% (9)	35% (17)	**0.1 (0.1, 0.1)**	**0.1 (0.1, 1.0)**
		HEU	15% (13)	27% (11)	0.1 (0.1, 0.1)	0.1 (0.1, 1.0)
	IgG	HIV+	11%	12%	0.1 (0.1, 0.5)	0.1 (0.1, 0.1)
		HEU	23%	9%	0.1 (0.1, 1.0)	0.1 (0.1, 0.1)
T-cell	IFNγ	HIV+	27% (11)	33% (12)	0.1 (0.1, 0.3)	0.1 (0.1, 0.5)
		HEU	36% (11)	43% (7)	0.1 (0.1, 0.8)	0.1 (0.1, 0.1)
	IL2	HIV+	55%	50%	0.5 (0.1, 1.0)	0.3 (0.1, 1.3)
		HEU	46%	29%	0.1 (0.1, 2.5)	0.1 (0.1, 1.0)

[a] N = number measured in each group.

[b] No significant differences in proportions of participants with ≥2-fold increases (i) between RV5- and placebo-recipients in HIV+ or HEU ($p>0.35$) or (ii) between HIV+ and HEU in RV5-recipients ($p>0.60$).

[c] Significantly higher PD3 IgA ELISPOT B-cell results in HIV+ RV5-recipients compared to placebo-recipients ($p=0.04$). No other significant differences in levels PD3 (i) between RV5- and placebo-recipients in HIV+ or HEU ($p>0.25$) or (ii) between HIV+ and HEU in RV5-recipients ($p>0.57$).

[d] Bold indicates significant differences.